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A taxonomic revision of the genus *Conidiobolus* (Ancylistaceae, Entomophthorales): four clades including three new genera

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Abstract

The genus *Conidiobolus* is an important group in entomophthoroid fungi and is considered to be polyphyletic in recent molecular phylogenies. To re-evaluate and delimit this genus, multi-locus phylogenetic analyses were performed using the large and small subunits of nuclear ribosomal DNA (nucLSU and nucSSU), the small subunit of the mitochondrial ribosomal DNA (mtSSU) and the translation elongation factor 1-alpha (EF-1α). The results indicated that the *Conidiobolus* is not monophyletic, being grouped into a paraphyletic grade with four clades. Consequently, the well-known *Conidiobolus* is revised and three new genera *Capillidium*, *Microconidiobolus* and *Neoconidiobolus* are proposed along with one new record and 22 new combinations. In addition, the genus *Basidiobolus* is found to be basal to the other entomophthoroid taxa and the genus *Batkoa* locates in the *Entomophthoraceae* clade.

Keywords

Zygomycetes, *Entomophthorales*, Morphology, Phylogeny, New taxa

Introduction

More than 250 species of entomophthoroid fungi were isolated from insects, soil and litter throughout the world (Gryganskyi et al. 2013). For a long time, this group has been considered to be polyphyletic (Nagahama et al. 1995; Jensen et al. 1998; James

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et al. 2006; Liu and Voigt 2010) and was classified into a subphylum *Entomophthoromycotina* and a pending taxon *Basidiobolus* (Hibbett et al. 2007). However, a recent phylogeny using the multi-gene dataset, 18S rDNA, 28S rDNA, mtSSU and RPB2, indicated that this group formed a monophyletic lineage including *Basidiobolus* and it was consequently reclassified as a new fungal phylum *Entomophthoromycota*. More recently, a phylogenomic analysis (192 clusters of orthologous proteins) has divided traditional zygomycotan into two phyla *Mucoromycota* and *Zoopagomycota* and the entomophthoroid fungi have been re-assigned into the subphylum *Entomophthoromycotina* under the latter phylum (Spatafora et al. 2016). This taxonomic scheme was supported by the phylogeny of mitochondrial genomes (Nie et al. 2019).

Together with other two genera *Ancylistes* and *Macrobiotophthora*, the genus *Conidiobolus* belongs to *Ancylistaceae*, *Entomophthorales*, *Entomophthoromycetes*, *Entomophthoromycotina* (Humber 2012). There are six and two accepted species within the *Ancylistes* and *Macrobiotophthora*, respectively, while *Conidiobolus*, one of the largest groups in the entomophthoroid fungi, contains 76 names (http://www.indexfungorum.org/). The genus *Conidiobolus* is typified by *C. utriculosus* Bref. 1884 and characterised morphologically by simple sporophores, globose to pyriform multinucleate primary conidia, various types of secondary conidia and resting spores (Brefeld 1884; Humber 1997). Up to the 1940s, for half a century, only three more species were reported, *C. minor* Bref., *C. villosus* Martin and *C. brefeldianus* Couch (Brefeld 1884; Martin 1925; Couch 1939). In the 1950s–1960s, 38 *Conidiobolus* species and a variety were isolated from the United States and India (Drechsler 1952, 1953a, b, 1954, 1955a, b, c, 1956, 1957a, b, c, 1960, 1961, 1962, 1965; Srinivasan and Thirumalachar 1961, 1962a, b, 1965, 1967, 1968a, b). Based on a numerical taxonomy, King (1976a, b, 1977) recognised 27 definitive species. Since then, along with some new combinations, 10 more species have been added to *Conidiobolus* (Bałazy et al. 1987; Waters and Callaghan 1989; Bałazy 1993; Huang et al. 2007; Waingankar et al. 2008; Nie et al. 2012, 2016, 2017, 2018). A total of 37 species are currently accepted in this genus (Nie et al. 2018).

Three subgenera – *Capillidium*, *Conidiobolus* and *Delacroixia* – were proposed within the *Conidiobolus*, based on shape of the secondary conidia and, amongst them, the subgenus *Delacroixia* was reduced from generic rank (Ben-Ze'ev and Kenneth 1982). This subgeneric criterion provided a valuable contribution for the taxonomy of the genus *Conidiobolus* (Humber 1989). Since the 1990s, molecular analysis has become an increasingly important tool for fungal taxonomy (Bruns et al. 1991; Taylor et al. 2000). The nucLSU rDNA and EF-1α regions proved to be distinguishable amongst *Conidiobolus* species (Nie et al. 2012), while nucSSU rDNA indicated the genus *Conidiobolus* might be a polyphyletic group (Jensen et al. 1998). The subgeneric circumscription was not defined because of instability to form a certain type of secondary conidia for each phylogenetic clade (Callaghan et al. 2000; Gryganskyi et al. 2013; Nie et al. 2018). Besides, the phylogenetic relationships amongst species of *Conidiobolus* have not been fully resolved due to the absence of types. The genus *Batkoa*, morphologically similar to *Conidiobolus*, was phylogenetically closely related to *Entomophthoraceae* rather than *Ancylistaceae* (Gryganskyi et al. 2012, 2013).

In the present study, a reclassification of the entomophthoroid fungi, including as many as available *Conidiobolus* types, was constructed based on four loci (nucSSU, nucLSU, EF-1α and mtSSU) to present the taxonomic delimitation of the genus *Conidiobolus* and to re-evaluate the phylogenetic relationship between *Basidiobolus* and *Batkoa*.

Materials and methods

Isolates and morphology

A total of 26 ex-types of *Conidiobolus* were purchased from the American Type Culture Collection, Manassas, USA (ATCC) and collected from the China General Microbiological Culture Collection Center, Beijing, China (CGMCC) and the Research Center for Entomogenous Fungi of Anhui Agricultural University, Anhui Province, China (RCEF). Dried cultures were deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS). Morphology was observed with an Olympus BX51 research microscope and photographed by an Olympus DP25 microscope-camera system. Growth diameter on PDA (potato 200 g, dextrose 20 g, agar 20 g, H_2O 1 l), Mycelia, primary conidiophores, primary conidia, microconidia, capilliconidia and resting spores were measured and described with the method of King (1976a).

DNA extraction, PCR amplification and sequencing

Fungal strains were incubated on PDA for 7 d at 21 °C. Total genomic DNA was extracted from the fresh fungal mycelia by using modified CTAB method (Watanabe et al. 2010). Four gene portions from cell nuclei and mitochondria and one protein coding gene were used in this study: the large subunit of nuclear ribosomal RNA genes (nucLSU), the small subunit of nuclear ribosomal RNA genes (nucSSU), the small subunit of mitochondrial ribosomal RNA genes (mtSSU) and the translation elongation factor 1-alpha gene (EF-1α). The nucLSU region was amplified with the primers LR0R and LR5 (Vilgalys and Hester 1990), the nucSSU region with nucSSU-0021-5' (Gargas and DePriest 1996) and nucSSU-1780-3' (DePriest 1993) and EF-1α region with the primers EF983 and EF1aZ-1R (http://www.aftol.org/primers.php). These PCR reactions have been described by Liu et al. (2005), Jensen et al. (1998) and Nie et al. (2012). The primers used for the mtSSU region were mtSSU1 and mtSSU2R and the PCR reaction was performed using the following cycling parameters: denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 1 min and finalised with an extra extension at 72 °C for 7 min (Zoller et al. 1999). PCR products were purified and sequenced by Shanghai Genecore Biotechnologies Company (Shanghai, China) with the same primers as relative PCR. The nucleotide sequence data have been deposited in the GenBank (Table 1).

Table 1. The species used in phylogenetic analyses.

Species	Strains*	GenBank accession numbers			
		nucSSU	nucLSU	EF-1α	mtSSU
Allomyces arbusculus	AFTOL 300	AY552524	DQ273806	DQ275334	–
Basidiobolus haptosporus	ARSEF 261	JX242606	JX242586	–	JX242626
B. heterosporus	CBS 311.66	JX242607	JX242587	–	JX242627
B. magnus	CBS 205.64	JX242608	JX242588	–	JX242628
B. meristosporus	CBS 931.73	JX242609	JX242589	–	JX242629
B. microsporus	CBS 130.62 (T)	JX242610	JX242590	–	JX242630
B. ranarum	NRRL 34594	AY635841	DQ273807	DQ275340	EF392490
Batkoa apiculata	ARSEF 3130	DQ177437	EF392404	–	EF392513
B. gigantea	ARSEF 214	JX242611	JX242591	–	JX242631
B. major	ARSEF 2936	EF392559	EF392401	–	EF392511
*B. obscurus***	CBS 182.60	JX242614	JX242595	–	JX242635
*B. pseudapiculata***	ARSEF 395	EF392557	EF392378	–	EF392508
Coemansia reversa	AFTOL 140	AY546685	AY546689	DQ282615	–
Conidiobolus adiaeretus	ARSEF 451 (T)	–	KC461182	–	–
C. adiaeretus	CGMCC 3.15888	–	MN061284	MN061481	MN061287
C. antarcticus	ARSEF 6913 (T)	–	DQ364207	–	DQ364227
C. bangalorensis	ARSEF 449 (T)	–	DQ364204	–	DQ364225
C. brefeldianus	ARSEF 452 (T)	AF368506	EF392382	–	EF392495
C. chlamydosporus	ATCC 12242 (T)	–	JF816212	JF816234	MK301178
C. coronatus	NRRL 28638	AF113418	AY546691	DQ275337	–
C. coronatus	RCEF 4518	–	JN131537	JN131543	–
C. couchii	ATCC 18152 (T)	–	JN131538	JN131544	MK301179
C. dabieshanensis	CGMCC 3.15763 (T)	–	KY398125	KY402206	MK301180
C. denaeosporus	ATCC 12940 (T)	–	JF816215	JF816228	MK301181
C. firmipilleus	ARSEF 6384	JX242612	JX242592	–	JX242632
C. gonimodes	ATCC 14445 (T)	–	JF816221	JF816226	MK301182
C. heterosporus	RCEF 4430	–	JF816225	JF816239	MK301183
C. humicolus	ATCC 28849 (T)	–	JF816220	JF816231	MK301184
C. incongruus	NRRL 28636	AF113419	AF113457	–	–
C. iuxtagenitus	ARSEF 6378 (T)	–	KC788410	–	–
C. iuxtagenitus	RCEF 4445	–	JX946695	JX946700	MK333391
C. khandalensis	ATCC 15162 (T)	–	KX686994	KY402204	MK301185
C. lachnodes	ARSEF 700	–	KC788408	–	–
C. lamprauges	ARSEF 2338	AF296754	DQ364206	–	DQ364226
C. lichenicolus	ATCC 16200 (T)	–	JF816216	JF816232	MK301186
C. lobatus	ATCC 18153 (T)	–	JF816218	JF816233	MK301187
C. marcosporus	ATCC 16578 (T)	–	KY398124	KY402209	MK301188
C. megalotocus	ATCC 28854 (T)	–	MF616383	MF616385	MK301189
C. mirabilis	CGMCC 3.17763 (T)	–	MH282852	MH282853	MK333389
C. mycophagus	ATCC 16201 (T)	–	JX946694	JX946698	MK301190
C. mycophilus	ATCC 16199 (T)	–	KX686995	KY402205	MK301191
C. nodosus	ATCC 16577 (T)	–	JF816217	JF816235	MK333388
C. osmodes	ARSEF 79	AF368510	EF392371	–	DQ364219
C. osmodes	RCEF4447	–	JN131539	JN131545	MK333392
C. pachyzygosporus	CGMCC 3.17764 (T)	–	KP218521	KP218524	MK333390
C. paulus	ARSEF 450 (T)	–	vv	–	–
C. polyspermus	ATCC 14444 (T)	–	MF616382	MF616384	MK301193
C. polytocus	ATCC 12244 (T)	–	JF816213	JF816227	MK301194
C. pumilus	ARSEF 453 (T)	JX242615	EF392383	–	EF392496
C. rhysosporus	ATCC 12588 (T)	–	JN131540	JN131546	MK301195
C. sinensis	RCEF 4952 (T)	–	JF816224	JF816238	MK301196
C. stilbeus	RCEF 5584 (T)	–	KP218522	KP218525	MK301197
C. stromoideus	ATCC 15430 (T)	–	JF816219	JF816229	MK301198

Species	Strains*	GenBank accession numbers			
		nucSSU	nucLSU	EF-1α	mtSSU
C. terrestris	ATCC 16198 (T)	–	KX752050	KY402208	MK301199
C. thromboides	ATCC 12587 (T)	–	JF816214	JF816230	MK301200
C. thromboides	FSU 785	JX242616	JX242597	–	JX242637
C. thromboides	RCEF 4492	–	JF816223	JF816236	MK333393
C. undulatus	ATCC 12943 (T)	–	JX946693	JX946699	MK301201
Dimargaris bacillispora	AFTOL 136	AB016020	DQ273791	DQ282609	–
Endogone pisiformis	AFTOL 539	DQ322628	DQ273811	DQ282618	–
Entomophaga aulicae	ARSEF 172	EF392542	EF392372	–	EF392487
E. conglomerata	ARSEF 2273	AF368509	–	–	–
E. maimaga	ARSEF 1400	EF392556	EF392395	–	EF392505
Eryniopsis caroloniana	ARSEF 640	EF392552	EF392387	–	EF392500
Entomophthora chromaphidis	ARSEF 1860	AF353725	–	–	–
E. culicis	ARSEF 387	AF368516	–	–	–
E. grandis	ARSEF 6701	–	DQ481229	–	–
E. scatophaga	ARSEF 6704	–	DQ481226	–	–
E. muscae	ARSEF 3074	AY635820	DQ273772	DQ275343	–
E. planconiana	ARSEF 6252	AF353723	GQ285878	–	–
E. schizophorae	ARSEF 5348	AF052402	GQ285883	–	–
E. syrphi	ARSEF 5595	–	DQ481230	–	–
E. tripidium	ARSEF 6518	AF296755	–	–	–
Erynia conica	ARSEF 1439	AF368513	EF392396	–	EF392506
E. ovispora	ARSEF 400	JX242620	JX242601	–	JX242641
E. rhizospora	ARSEF 1441	AF368514	EF392397	–	EF392507
E. sciarae	ARSEF 1870	AF368515	EF392399	–	EF392509
Furia americana	ARSEF 742	EF392554	EF392389	–	–
F. gastropachae	ARSEF 5541	EF392562	EF392407	–	EF392516
F. ithacensis	ARSEF 663	EF392553	EF392388	–	EF392501
F. neopyralidarum	ARSEF 1145	AF368518	EF392394	–	EF392504
F. pieris	ARSEF 781	AF368519	EF392390	–	EF392502
F. virescens	ARSEF 1129	EF392555	EF392393	–	EF392503
Gaertneriomyces semiglobiferus	AFTOL 34	AF164247	DQ273778	DQ275338	–
Macrobiotophthora vermicola	ARSEF 650	AF052400	–	–	–
Massospora cicadina	ARSEF 374	EF392548	EF392377	–	EF392492
Mortierella verticillata	AFTOL 141	AF157145	DQ273794	–	–
Pandora blunckii	ARSEF 217 (T)	JX242621	JX242602	–	–
P. delphacis	ARSEF 459	AF368521	EF392384	–	EF392497
P. dipterigena	ARSEF 397	AF368522	EF392380	–	EF392565
P. kondoiensis	CBS 642.92	JX242622	JX242603	–	JX242642
P. neoaphidis	ARSEF 3240	EF392560	EF392405	–	EF392514
Piptocephalis corymbifera	AFTOL 145	AB016023	AY546690	DQ282619	–
Rhizophagus intraradices	AFTOL 845	DQ322630	FJ461839	DQ282611	–
Rozella allomycis	AFTOL 297	AY635838	DQ273803	DQ275342	–
Schizangiella serpentis	ARSEF 2237	AF368523	EF392428	–	EF392488
Strongwellsea castrans	–	AF052406	–	–	–
Zancudomyces culisetae	AFTOL 29	AF277007	DQ273773	–	–
Zoophthora anglica	ARSEF 396	–	EF392379	–	EF392493
Z. lanceolata	ARSEF 469	EF392550	EF392385	–	EF392498
Z. phalloides	ARSEF 2281	EF392558	EF392400	–	EF392510
Z. radicans	ARSEF 388	JX242624	JX242605	–	JX242644

* AFTOL, Assembling the Fungal Tree of Life; ARSEF, ARS Entomopathogenic Fungus Collection (Ithaca, U.S.A.); ATCC, American Type Culture Collection (Manassas, U.S.A); CGMCC, China General Microbiological Culture Collection Center (Beijing, China); FSU, Jena Microbial Resource Collection (Friedrich-Schiller-University of Jena, Germany); NRRL, ARS Culture Collection (Peoria, U.S.A); RCEF, Research Center for Entomogenous Fungi (Hefei, China). T = ex-type.
** *Batkoa* sp. CBS 182.60 was received as *Conidiobolus obscurus,* while *B. pseudapiculata* ARSEF 395 was received as *C. pseudapiculatus*.

Phylogenetic analyses

More available nucLSU, nucSSU, mtSSU and EF-1α sequences of 14 *Conidiobolus* species and 47 other entomophthoroid fungi were obtained from GenBank. Ten species of *Glomeromycotina*, *Mortierellomycotina*, *Mucoromycotina*, *Kickxellomycotina*, *Zoopagomycotina*, *Blastocladiomycota*, *Chytridiomycota* and *Cryptomycota*, were chosen as outgroups. Alignments were constructed separately for each locus with MUSCLE 3.8.31 (Edgar 2004) and the concatenated matrices were assembled by SequenceMatrix 1.7.8 (Vaidya et al. 2011). The best model for the phylogenetic analysis was selected with Akaike Information Criterion (AIC) by using Modeltest 3.7 (Posada and Crandall 1998). Phylogenetic relationships were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI). The best-scoring ML tree analysis was performed using raxmlGUI 1.5b1 with GTRGAMMA model and 1000 replicates (Silvestro and Michalak 2012). The BI analysis was performed using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003). Markov Chain Monte Carlo (MCMC) chains ran until the convergences met and the standard deviation fell below 0.01. The first 25% of trees were discarded as burn-in. The combined dataset was deposited at TreeBase (No. S25064). Phylogenetic trees were checked and modified in FigTree 1.4 (Rambaut 2012).

Results

Phylogenetic analyses

The combined dataset contained 4521 characters of nucLSU (1–1326), nucSSU (1327–3424), EF-1α (3425–4062) and mtSSU (4063–4521) after alignment. With the optimal model GTR+I+G and random starting trees, four Markov chains were run for 7 million generations and every 100th generation was sampled once. ML and BI analyses of the combined dataset resulted in phylogenetic reconstructions with almost similar topologies and the average standard deviation of split frequencies was 0.006721 (BI).

In the ML phylogenetic tree (Figure 1), the *Basidiobolaceae* lineage (88/0.94) is located at the base of the entomophthoroid fungi and is closely related to the *Ancylistaceae* group (56/0.91). The *Batkoa* lineage is grouped within the *Entomophthoraceae* Clade (60/0.89). All *Conidiobolus* lineages are clustered into a paraphyletic grade and therefore cannot be considered congeneric. Moreover, the *Conidiobolus* grade consists of four well supported clades. In detail, there are 7, 10, 16 and 3 species in Clade I (100/1.00), II (77/1.00), III (100/1.00) and IV (99/1.00), respectively.

Taxonomy

In order to provide a more natural taxonomic classification, four genera (*Capillidium*, *Conidiobolus*, *Microconidiobolus* and *Neoconidiobolus*) and their type species (*Ca. heterosporum*, *C. utriculosus*, *M. paulus* and *N. thromboides*) are described here in this paper.

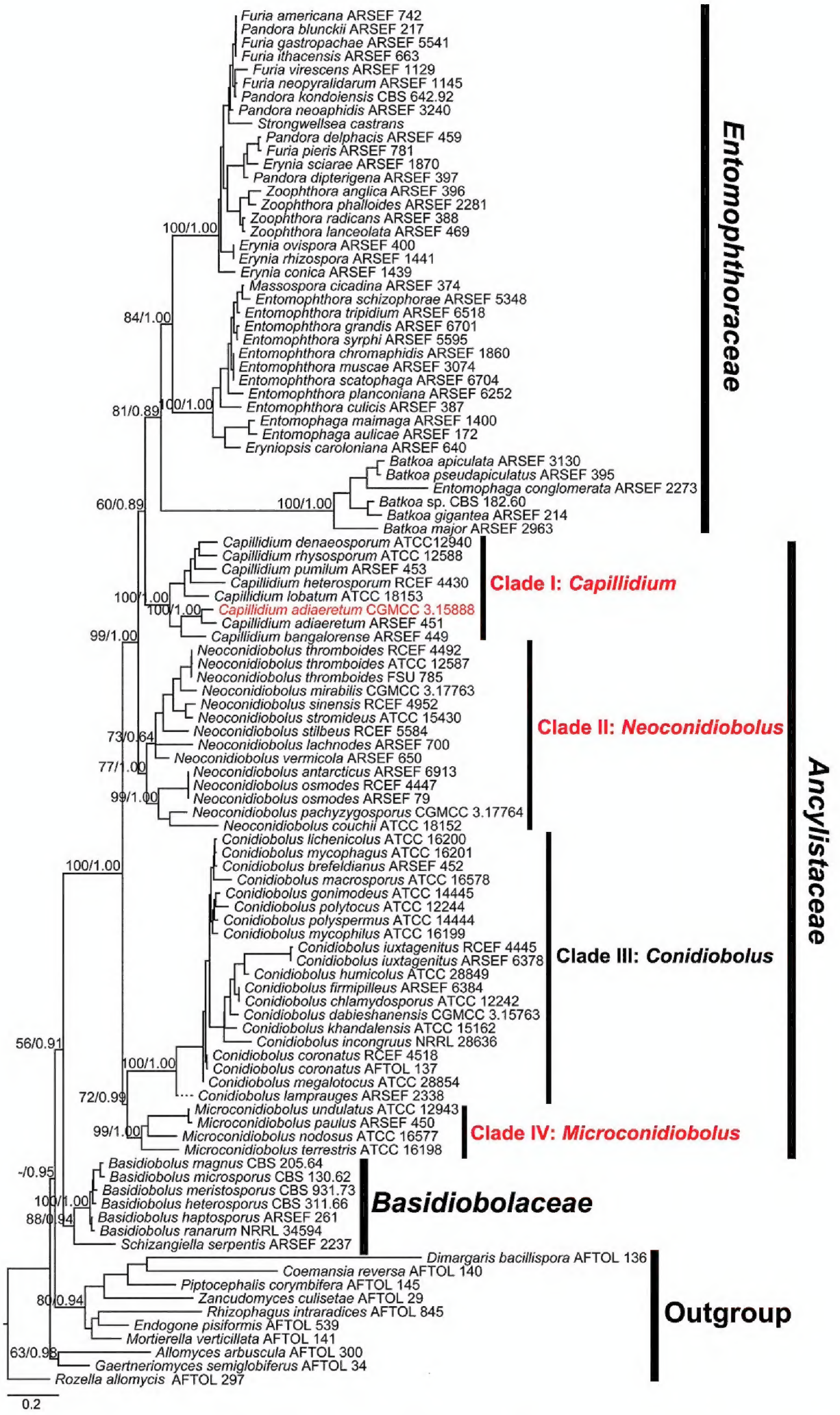

Figure 1. Phylogenetic tree constructed by maximum likelihood analyses of nucLSU, nucSSU, EF-1α and mtSSU sequences for *Entomophthoromycotina*, with some chytrid and mucoralean fungi as outgroups. Three new genera and one Chinese new record are shown in red. Maximum likelihood bootstrap values (≥ 50%) / Bayesian posterior probabilities (≥ 0.50) of main clades are indicated along branches. Scale bar indicates substitutions per site.

Additionally, a new record *Ca. adiaeretum*, *C. coronatus* and *C. iuxtagenitus* with new isolates from China and *C. khandalensis* being first reported to produce microconidia are illustrated herein.

Capillidium B. Huang & Y. Nie, gen. nov.

MycoBank No: MB831596

Etymology. Referring to unique ellipsoidal secondary conidia (capilliconidia).

Type species. *Capillidium heterosporum* (Drechsler) B. Huang & Y. Nie.

Description. Mycelia colourless. Primary conidiophores simple, bearing a single primary conidia. Primary conidia forcibly discharged multinucleate, colourless, globose, pyriform to obovoid. Two kinds of replicative conidia, the first one is similar and smaller than primary conidia, the second one (capilliconidia) arises from elongate and slender conidiophores. Zygospores present or absent, formed in axial alignment with conjugating segments, globose to subglobose, often smooth, sometimes rough, colourless or yellowish.

Notes. *Conidiobolus* subgen. *Capillidium* Ben-Ze'ev & Kenneth was firstly established to include species with capilliconidia (Ben-Ze'ev and Kenneth 1982). In this phylogenetic analysis, all members of the subgenus *Capillidium* grouped with good support (100/1.00) and, therefore, it was raised from subgenus to genus status based on the monophyly, as well as the stability to form ellipsoidal secondary conidia (capilliconidia). In addition to capilliconidia, *C. adiaeretus* also produces microconidia.

Capillidium heterosporum (Drechsler) B. Huang & Y. Nie, comb. nov.

MycoBank No: MB831601

Figure 2

Conidiobolus heterosporus Drechsler, Am. J. Bot. 40: 107 (1953). Basionym.
=*Conidiobolus rugosus* Drechsler, Am. J. Bot. 42: 437 (1955).

Specimens examined. China, Anhui Province, Plant detritus, 8 Nov 2008, *C.F. Wang, RCEF 4430*.

Description. Colonies on PDA at 25 °C after 3 d, white, reaching ca. 21 mm in diameter. Mycelia colourless, 5–9 μm wide. Primary conidiophores, colourless, unbranched and producing a single globose conidium with widening upwards, extending to a length of 30–245 μm into the air, 8–17 μm wide. Primary conidia forcibly discharged, colourless, globose to subglobose, measuring 12–37 μm in greatest length and 11–31 μm in total width, including a basal papilla 1.5–5 μm high and 5–12 μm wide. After discharging on to 2% water-agar, similar and smaller secondary conidia arise from primary conidia, 1–6 ellipsoidal secondary conidia (capilliconidia, 10–20 × 12–38 μm) arise from slender conidiophores (50–250 × 2.5–4 μm). Resting spores not observed.

Notes. The ex-type living culture is ATCC 12941 (United States, Maryland, 18 Mar 1952, Drechsler).

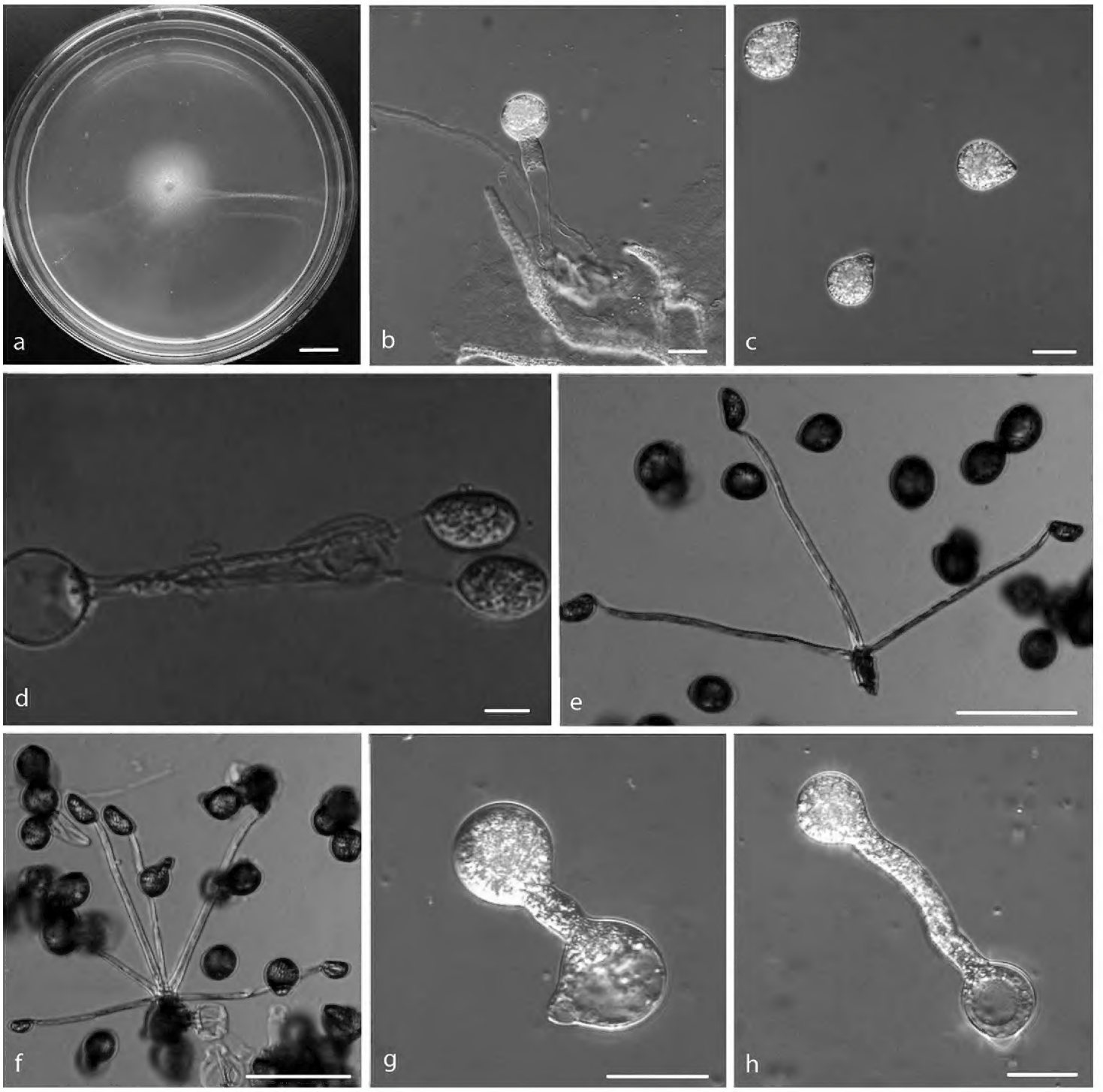

Figure 2. *Capillidium heterosporum* **a** colony on PDA after 3 d at 25 °C **b** primary conidiophores bearing primary conidia **c** primary conidia **d, e, f** ellipsoidal secondary conidia arising from slender conidiophores **g, h** production of secondary conidia. Scale bars: 10 mm (**a**); 20 μm (**b, c, d, g, h**); 100 μm (**e, f**).

Capillidium adiaeretum **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831602
Figure 3

Conidiobolus adiaeretus Drechsler, J. Wash. Acad. Sci. 43: 42 (1953). Basionym.

Specimens examined. China, Jiangsu Province, Nanjing City, Laoshan Forest Park, 32°5'58"N, 118°35'53"E, Plant detritus, 1 Dec 2018, *Y. Nie and Y. Gao, HMAS 248358*, culture *CGMCC 3.15888 (=RCEF 6550)*.

Description. Colonies on PDA at 25 °C after 3 d, white, reaching ca. 7–10 mm in diameter. Mycelia colourless, 3–4.5 μm wide. Primary conidiophores, colourless, unbranched and producing a single globose conidium with widening upwards; they offer

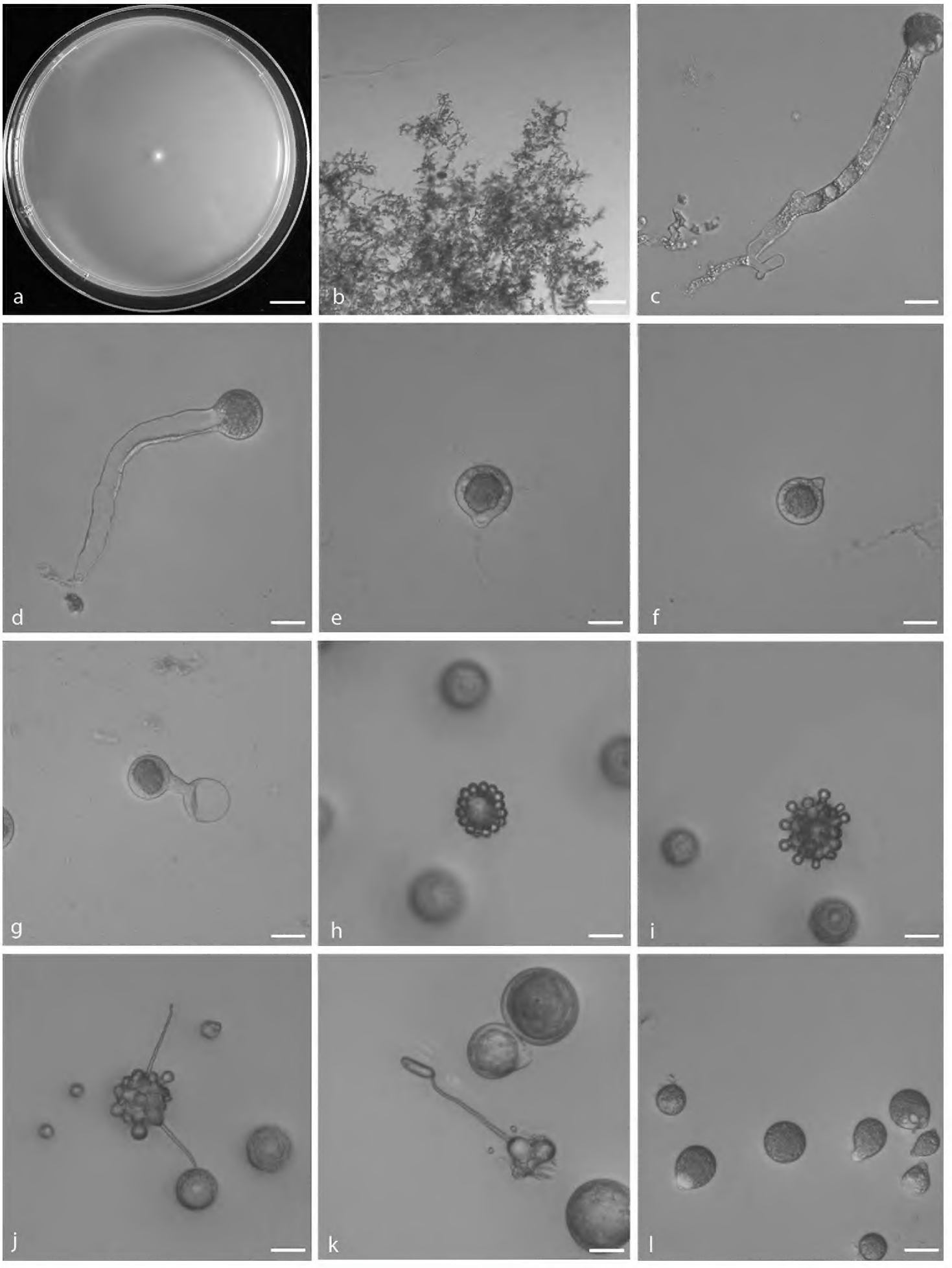

Figure 3. *Capillidium adiaeretum* **a** colony on PDA after 3 d at 25 °C **b** mycelia **c, d** primary conidiophores bearing primary conidia **e, f** primary conidia **g** Production of secondary conidia **h** first stage of forming microconidia **i** second stage of forming microconidia **j, k** ellipsoidal secondary conidia arising from slender conidiophores **l** chlamydospores. Scale bars: 10 mm (**a**); 100 μm (**b**); 20 μm (**c–l**).

a pronounced dimensional contrast with the mycelial filaments, extending to a length of 50–210 μm into the air, 3–25 μm wide. Primary conidia forcibly discharged, colourless, globose, measuring 15–45 μm in greatest length and 13–42 μm in total width, including a basal papilla 2–6 μm high and 5–17 μm wide. After discharging on to 2% water-agar, similar and smaller secondary conidia arise from primary conidia, two generations of multiple spherical units forming on the parent globose conidia Microconidia only formed from the second set, 5–12 × 9–10 μm. Capilliconidia formed readily from discharged microconidia, 16–24 × 5–6 μm. Chlamydospores formed within the substratum, colourless, globose to ellipsoidal, 13–40 × 15–45 μm.

Notes. The species was firstly reported from America (Drechsler 1953a). The ex-type living culture is ATCC 12589 isolated by Drechsler (1953a). It is mainly characterised and differs from other *Capillidium* species by its ability to form both microconidia and capilliconidia (Callaghan et al. 2000). The Chinese specimen CGMCC 3.15888 clusters completely (100/1.00) with an isotype ARSEF 451 (98% sequence similarity in nucLSU) and fits well with its morphological descriptions. It is reported in China for the first time.

Conidiobolus **Bref., Mykol. Untersuch. 6(2): 35 (1884), emend.**
MycoBank No: MB20144

= *Delacroixia* Sacc. & P. Syd., Syll. fung. (Abellini) 14(1): 457 (1899).
Conidiobolus subgen. *Delacroixia* (Sacc. & P. Syd.) Tyrrell & Macleod, J. Invert. Pathol. 20: 12 (1972).

Type species. *Conidiobolus utriculosus* Bref.

Description. Mycelia colourless. Primary conidiophores simple or branched dichotomously, positively phototropic, bearing a single or 2–4 primary conidia. Primary conidia forcibly discharged, multinucleate, colourless, pyriform, obovoid, globose to subglobose. Secondary conidia usually with shape of primary conidia but smaller, formed singly on short secondary conidiophores. Microspores arising from primary or secondary conidia. Villose appendaged globose conidia and formed villose conidia. Chlamydospores formed intercalarily within assimilative hyphae. Zygospores formed in axial alignment with one or two (homothallic or heterothallic) conjugating segments.

Notes. *C. utriculosus*, the type species of the genus *Conidiobolus*, has not been re-collected since Brefeld isolated it in 1884 and most taxonomists working on entomophthoroid fungi now universally recognised it as *C. coronatus* (Gryganskyi et al. 2013). However, the smaller pear-shaped conidia of *C. utriculosus* are different from the larger globose conidia of *C. coronatus* and villose spores in *C. coronatus* are not observed in *C. utriculosus* (Brefeld 1884; King 1977). Consequently, *C. coronatus* is not synonymised with *C. utriculosus* in this study. Instead, this study

agrees with Srinivasan and Thirumalachar (1967) and King (1977) to place *C. minor* in synonymy with *C. utriculosus* because the small conidia of *C. minor* were probably replicative conidia of *C. utriculosus*. Nevertheless, neither *C. utriculosus* nor *C. minor* has available living cultures. Therefore, we have not yet designated an epitype and thus no DNA sequences for explaining this type. Fortunately, we are able to recognise clade III (Fig. 1) as *Conidiobolus* on the basis of its synapomorph, namely microspores.

***Conidiobolus utriculosus* Bref., Mykol. Untersuch. 6(2): 35 (1884)**
MycoBank No: MB144259 (MBT391377)

= *Conidiobolus minor* Bref., Mykol. Untersuch. 6(2): 35, 68 (1884).

Specimens examined. No ex-type.

Description. Refer to Brefeld (1884) and King (1977).

Notes. Due to the lack of ex-type, plates 3, 4, and 5 in Brefeld, Mykol. Untersuch. 6(2): 35 (1884) are designated here as the lectotype for *Conidiobolus utriculosus*.

***Conidiobolus coronatus* (Costantin) A. Batko, Entomophaga, Mémoires hors série 2: 129 (1964)**
MycoBank No: MB283037
Figure 4

Boudierella coronata Costantin, Bull. Soc. mycol. Fr. 13: 40 (1897). Basionym.
Delacroixia coronata (Costantin) Sacc. & P. Syd., Syll. fung. (Abellini) 14(1): 457 (1899).
Entomophthora coronata (Costantin) Kevorkian, J. Agric. Univ. Puerto Rico 21(2): 191 (1937).
= *Conidiobolus villosus* G.W. Martin, Bot. Gaz. 80(3): 317 (1925).

Specimens examined. China, Shandong Province, Plant detritus, 20 Mar 2009, *C.F. Wang, RCEF 4518*.

Description. Colonies grown on PDA for 3 d at 21 °C, reaching ca. 65 mm in diameter. Mycelia colourless, 8–20 μm wide. Primary conidiophores, positively phototropic, colourless, unbranched and producing a single globose conidium, extending to a length of 53–287 μm into the air, 7.5–20.5 μm wide. Primary conidia forcibly discharged, colourless, globose, measuring 36–52 μm in greatest width and 42–65 μm in total length, including a basal papilla 12–18 μm high and 6.5–14 μm wide. After discharging on to 2% water-agar, similar and smaller secondary conidia arise from primary conidia. Microconidia produced readily from primary conidia, globose or almond-shaped, 13–19 × 11–15 μm. Villose spores formed after 4–5 d, globose, 20–42 μm.

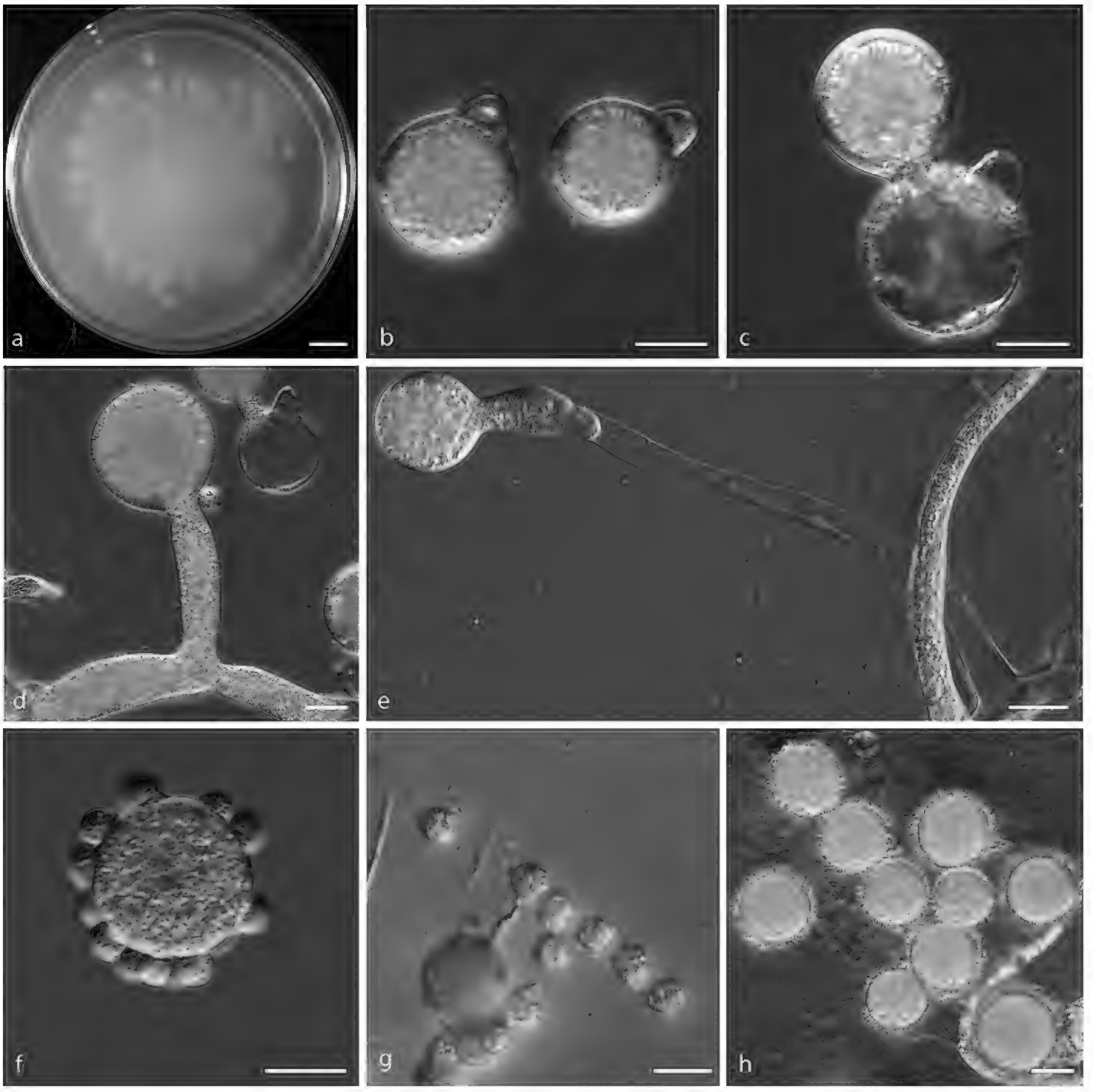

Figure 4. *Conidiobolus coronatus* **a** colony on PDA after 3 d at 21 °C **b** primary conidia **c** production of secondary conidia **d, e** primary conidiophores bearing primary conidia **f, g** microconidia **h** villose spores. Scale bars: 10 mm (**a**); 20 μm (**b–h**).

Notes. The ex-type living culture is ATCC 28691 (United States, Louisiana, Plant detritus, 3 January 1972). Due to the absence of molecular data of ex-type strain ATCC 28691, the molecular data of the authentic strain NRRL 28638, which has been applied in many other phylogenetic analysis (James et al. 2006; Liu and Voigt 2011; Gryganskyi et al. 2012; Tretter et al. 2014; Spatafora et al. 2016), was used in this study instead. The monotypic genus *Delacroixia* was typified by *D. coronata* which was transferred from an ascomycete *Boudierella coronata* Costantin (Costantin 1897; Saccardo and Sydow 1899). After that, it was reclassified as a subgenus of *Conidiobolus*, namely *Conidiobolus* sub. *Delacroixia* (Sacc. & P. Syd.) Tyrrell & MacLeod to define all those *Conidiobolus* species capable of forming microspores and, consequently, *D. coronata* was recombined as *C. coronatus* (Tyrrell and MacLeod 1972; Ben-Ze'ev and Kenneth 1982).

Conidiobolus iuxtagenitus **S.D. Waters & Callaghan, Mycol. Res. 93(2): 223 (1989)**
MycoBank No: MB135617
Figure 5a–g

Specimens examined. China, Anhui Province, Plant detritus, 8 Nov 2008, *C.F. Wang, RCEF 4445*.

Description. Colonies on PDA at 21 °C after 3 d white, flat, slow-growing, reaching ca. 13 mm in diameter. Mycelia colourless, 5.5–11 μm wide. Primary conidiophores, positively phototropic, arising from hyphal segments, colourless, 28–75 × 7.5–10 μm, unbranched and producing a single globose conidium. Primary conidia forcibly discharged, globose, 27–37 × 21–28 μm, with a basal papilla 6–10 μm wide. Secondary conidia arising from primary conidia, similar to, but smaller than the primary ones, forcibly discharged. Tertiary conidium fusiform arising from primary conidia, 30–45 × 16–22 μm. Zygospores in a position separated by a short beak near a lateral conjugation, globose to subglobose, smooth, 21–25 × 17–24 μm, with a 1–2 μm thick wall.

Notes. The ex-type living culture is ARSEF 6378 (United Kingdom, Staffordshire, Plant detritus, 31 October 1983, M. F. Smith).

Conidiobolus khandalensis **Sriniv. & Thirum., Mycologia 54(6): 692 (1963) [1962]**
MycoBank No: MB328754
Figure 5h

Specimens examined. India, Khandala, Dec. 1961, *Srinivasan and Thirumalachar, ATCC 15162*.

Description. Refer to Srinivasan and Thirumalachar (1962b). Microconidia produced from global conidia on the 2% water-agar at 16 °C (Fig. 5h).

Notes. According to the original morphological description (Srinivasan and Thirumalachar 1962b) and the re-examination by King (1977), microconidia have not been reported. However, we observed the microconidia produced from global conidia on 2% water-agar at 16 °C. Moreover, this specimen was located in the *Conidiobolus* lineage (Figure 1) which was supported by our morphological analyses.

Microconidiobolus **B. Huang & Y. Nie, gen. nov.**
MycoBank No: MB831597

Etymology. Referring to small discharged primary conidia.

Type species. *Microconidiobolus paulus* (Drechsler) B. Huang & Y. Nie.

Description. Mycelia colourless. Primary conidiophores simple and short, bearing a single primary conidia. Primary conidia forcibly discharged, multinucleate, colourless, globose to obovoid, usually small, mostly less than 20 μm. Only globose repli-

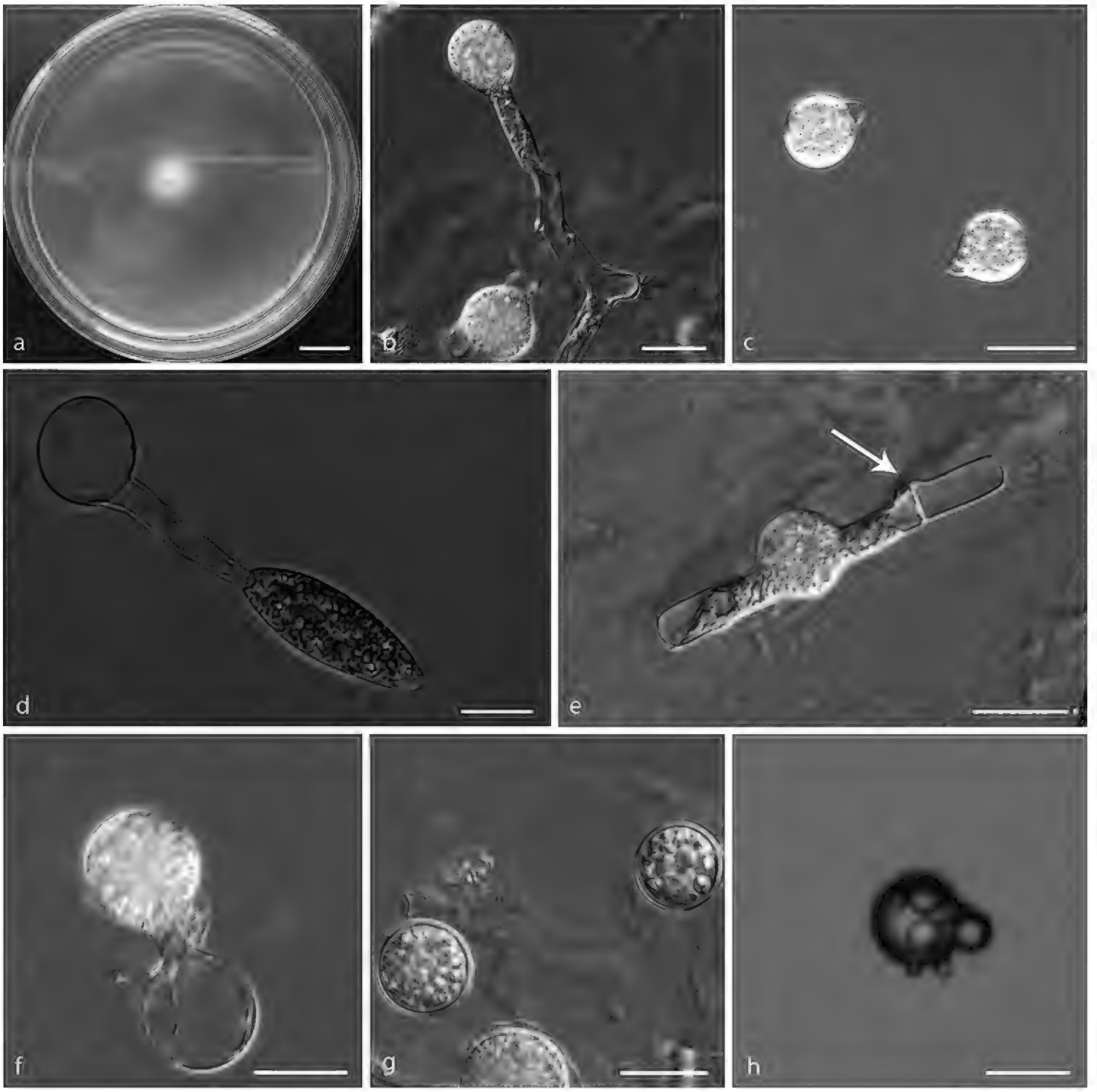

Figure 5. a–g *Conidiobolus iuxtagenitus* **h** *Conidiobolus khandalensis* **a** colony on PDA after 3 d at 21 °C **b** primary conidiophores bearing primary conidia **c** primary conidia **d** tertiary fusiform conidium from a globose spore **e** zygospore formation with the beak almost emptied of protoplasm **f** production of secondary conidia **g** zygospores **h** microconidia produced from global conidia. Scale bars: 10 mm (**a**); 20 μm (**b–h**).

cative conidia produced, similar and smaller than primary conidia. Chlamydospores globose, formed terminally on hyphae or from globose cells by thickening of the wall. Zygospores formed in axial alignment with two conjugating segments, globose to ellipsoidal, smooth and yellowish.

Notes. This genus includes three species producing smaller primary conidia (mostly less than 20 μm) without microspores or capilliconidia compared to other *Conidiobolus* spp. These three species are *C. nodosus*, *C. paulus* and *C. terrestris*. According to the taxonomic scheme of *Conidiobolus* by King (1977), *C. undulatus* is a synonym of *C. paulus*, which is supported herein by molecular evidence (Figure 1). However, the phylogeny does not support *C. nodosus* and *C. terrestris* as synonyms of *C. lachnodes*, since the former two were located in clade IV and the latter in clade II

(Figure 1). Therefore, we accept the taxonomic status at species level for *C. nodosus* and *C. terrestris*, based on the morphological and phylogenetic analyses.

Microconidiobolus paulus **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831605

Conidiobolus paulus Drechsler, Bull. Torrey bot. Club. 84: 269 (1957). Basionym.
= *Conidiobolus undulatus* Drechsler, Bull. Torrey bot. Club. 84: 275 (1957).
= *Conidiobolus parvus* Drechsler, Bull. Torrey bot. Club. 89: 233 (1962).

Description. Refer to Drechsler (1957a).

Notes. The ex-type living culture is ATCC 12942 (United States, Wisconsin, 18 November 1954, Drechsler).

Neoconidiobolus **B. Huang & Y. Nie, gen. nov.**
MycoBank No: MB831598

Etymology. Referring to the subgenus *Conidiobolus* raised to generic rank.

Type species. *Neoconidiobolus thromboides* (Drechsler) B. Huang & Y. Nie.

Description. Mycelia colourless. Primary conidiophores simple, sometimes branched from hyphal knots or differentiated from aerial hyphae, positively phototropic, bearing a single primary conidium. Primary conidia forcibly discharged, multinucleate, colourless, globose, pyriform to obovoid. Replicative conidia similar and smaller than primary conidia. Chlamydospores globose, formed terminally on hyphae or from globose cells by thickening of the wall. Zygospores formed in axial alignment with two conjugating segments, globose to ellipsoidal, smooth, colourless, rarely pale yellowish.

Notes. The genus *Neoconidiobolus* is strikingly similar to the subgenus *Conidiobolus* which produces neither microconidia nor capilliconidia. All members in the clade of *Neoconidiobolus* share the following characteristics: forcibly discharged, colourless, globose, pyriform to obovoid primary conidia. Two kinds of replicative conidia produced. One is discharged, similar and smaller than primary conidia and the other is elongate and forcibly discharged. Two types of resting spores produced: zygospores and chlamydospores.

Neoconidiobolus thromboides **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831606
Figure 6

Conidiobolus thromboides Drechsler, J. Wash. Acad. Sci. 43: 38 (1953). Basionym.

Specimens examined. China, Anhui Province, Plant detritus, 21 Feb 2009, *C.F. Wang, RCEF 4492*.

Description. Colonies grown on PDA for 3 d at 25 °C, white, reaching ca. 30 mm diameter. Mycelium colourless, filamentous, 5–7.5 µm wide. Primary conidiophores colourless, unbranched and producing a single conidium, 50–122.5 × 6–16.5 µm. Primary conidia forcibly discharged, colourless, globose to subglobose, 20–26.5 µm wide, 26.5–34 µm long, including a basal papilla 6–10 µm wide. Secondary conidia globose, forming from the primary conidia. Zygospores most often formed between segments of separate hyphae. Mature zygospores smooth, globose to subglobose, 25–30 µm in diameter with wall 2–3 µm thick.

Notes. The ex-type living culture is ATCC 12587 (United States, New Hampshire, September 1957, Drechsler).

More new combinations

In addition to previously described taxa, more new combinations were proposed herein and their descriptions refer to relevant protologues.

Capillidium bangalorense **(Sriniv. & Thirum.) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831607

Conidiobolus bangalorensis Sriniv. & Thirum., Mycologia 59(4): 702 (1967). Basionym.

Capillidium denaeosporum **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831608

Conidiobolus denaeosporus Drechsler, J. Wash. Acad. Sci. 47: 309 (1957). Basionym.

Capillidium lobatum **(Sriniv. & Thirum.) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831609

Conidiobolus lobatus Sriniv. & Thirum., J. Elisha Mitchell scient. Soc. 84: 212 (1968). Basionym.

Capillidium pumilum **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831610

Conidiobolus pumilus Drechsler, J. Wash. Acad. Sci. 45: 115 (1955). Basionym.
= *Conidiobolus globuliferus* Drechsler, Am. J. Bot. 43: 783 (1957) [1956].
= *Conidiobolus inordinatus* Drechsler, J. Wash. Acad. Sci. 47: 312 (1957).

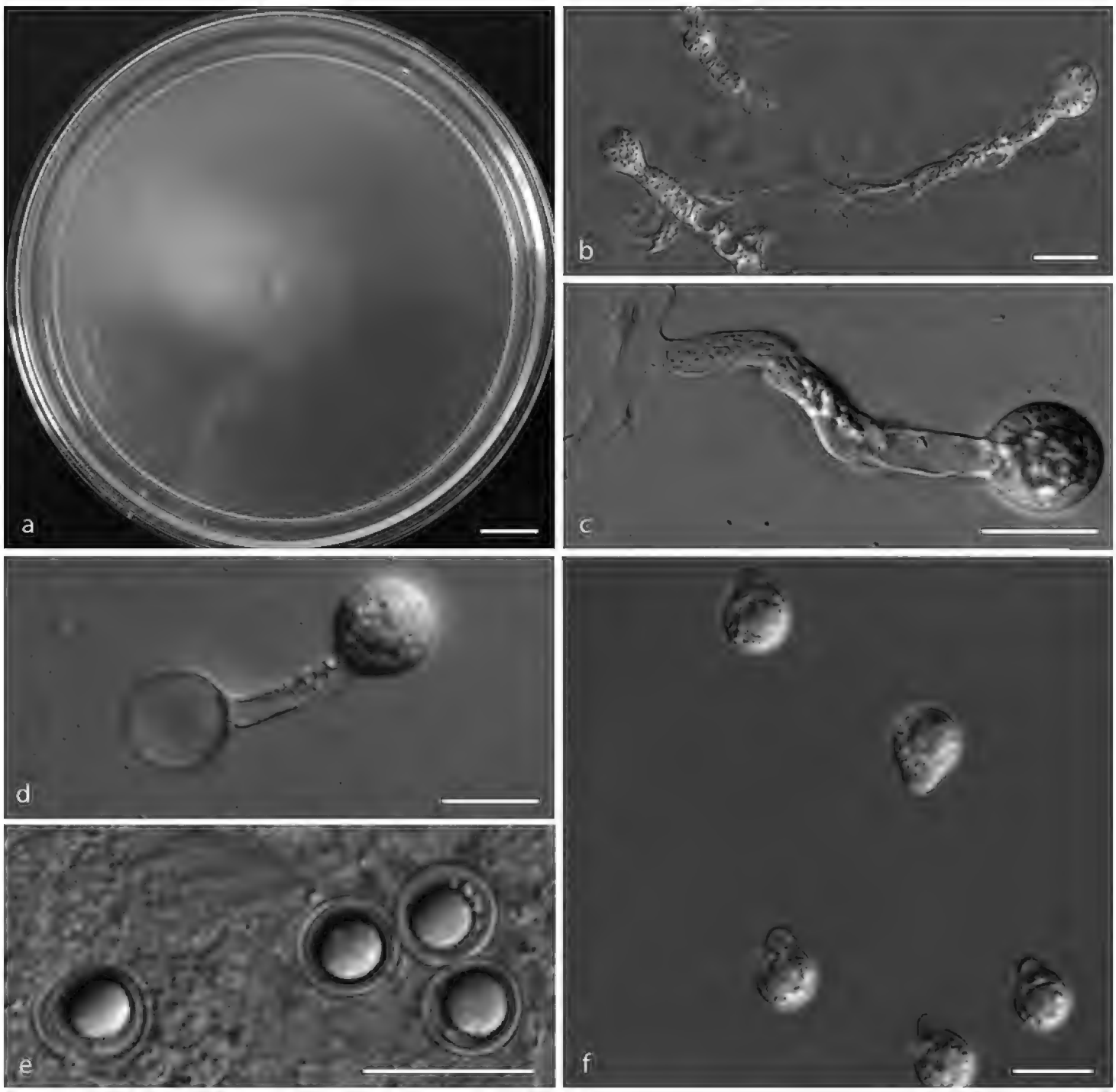

Figure 6. Neoconidiobolus thromboides **a** colony on PDA after 3 d at 25 °C **b, c** primary conidiophores bearing primary conidia **d** production of secondary conidia **e** zygospores **f** primary conidia. Scale bars: 10 mm (**a**); 20 μm (**b–d, f**); 40 μm (**e**).

Capillidium rhysosporum **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831611

Conidiobolus rhysosporus Drechsler, Am. J. Bot. 41: 567 (1954). Basionym.

Microconidiobolus nodosus **(Sriniv. & Thirum.) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831624

Conidiobolus nodosus Sriniv. & Thirum., Mycologia 59(4): 705 (1967). Basionym.

Microconidiobolus terrestris **(Sriniv. & Thirum.) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831625

Conidiobolus terrestris Sriniv. & Thirum., Mycopathol. Mycol. appl. 36(3–4): 344 (1968). Basionym.

Neoconidiobolus couchii **(Sriniv. & Thirum.) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831626

Conidiobolus couchii Sriniv. & Thirum., J. Elisha Mitchell scient. Soc. 84: 211 (1968). Basionym.

Neoconidiobolus lachnodes **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831627

Conidiobolus lachnodes Drechsler, Am. J. Bot. 42: 442 (1955). Basionym.

Neoconidiobolus mirabilis **(Y. Nie & B. Huang) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831628

Conidiobolus mirabilis Y. Nie & B. Huang, Mycol. Progr. 17(10): 1204 (2018). Basionym.

Neoconidiobolus osmodes **(Drechsler) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831629

Conidiobolus osmodes Drechsler, Am. J. Bot. 41: 571 (1954). Basionym.
= *Conidiobolus antarcticus* S. Tosi, Caretta & Humber, Mycotaxon 90(2): 344 (2004).

Neoconidiobolus pachyzygosporus **(Y. Nie & B. Huang) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831630

Conidiobolus pachyzygosporus Y. Nie & B. Huang, Mycol. Progr. 17(10): 1206 (2018). Basionym.

Neoconidiobolus sinensis **(Y. Nie, X.Y. Liu & B. Huang) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831631

Conidiobolus sinensis Y. Nie, X.Y. Liu & B. Huang, Mycotaxon 120: 432 (2012). Basionym.

Neoconidiobolus stilbeus **(Y. Nie & B. Huang) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831632

Conidiobolus stilbeus Y. Nie & B. Huang, Mycosphere 7(6): 804 (2016). Basionym.

Neoconidiobolus stromoideus **(Sriniv. & Thirum.) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831633

Conidiobolus stromoideus Sriniv. & Thirum., Sydowia 16(1–6): 65 (1963) [1962]. Basionym.

Neoconidiobolus vermicola **(J.S. McCulloch) B. Huang & Y. Nie, comb. nov.**
MycoBank No: MB831634

Entomophthora vermicola J.S. McCulloch, Trans. Br. mycol. Soc. 68(2): 173 (1977). Basionym.
Macrobiotophthora vermicola (J.S. McCulloch) B.E. Tucker, Mycotaxon 13(3): 499 (1981).

Discussion

The phylogenetic position of *Basidiobolus* in the Kingdom *Fungi* has been problematic for a long time. Previous phylogenetic analyses of the rDNA (18S, 28S and 5.8S) sequences grouped *Basidiobolus* outside or basal in the *Entomophthorales* (Nagahama et al. 1995; Jensen et al. 1998; White et al. 2006). Combined with the study of other protein-coding molecular markers, *Basidiobolus* was located inside the *Entomophthorales* (James et al. 2006). Recently, according to the phylogeny of much more available molecular data of entomophthoroid fungi in three families, *Basidiobolus* was grouped basal to other entomophthoroid taxa (Gryganskyi et al. 2012) which was also supported by the phylogenomic analyses of zygomycete fungi (Spatafora et al. 2016) and by the multi-gene analyses in this study. Although the morphological characteristics of *Batkoa* were similar to *Conidiobolus*, the *Batkoa* lineage appeared to be most closely related to the other taxa in the *Entomophthoraceae* Clade and should be distinguished

from *Conidiobolus* lineage by its obligate pathogenicity for invertebrates and by staining readily, while most members of *Conidiobolus* are saprobic and non-staining.

The phylogenetic relationship of the genus *Conidiobolus* has been unclear for a long time, because of its high heterology (Gryganskyi et al. 2013). This article used more available ex-type strains to revise this genus, based on phylogeny and morphology. According to Figure 1, four main clades were reconstructed and the results showed that *Conidiobolus* s.l. is not a monophyletic group but paraphyletic with *Macrobiotophthora vermicola*. The *M. vermicola* was originally placed in *Entomophthora* (Mcculloch 1977) and transferred to *Macrobiotophthora*, based on the morphological characters of primary spores, secondary spores and zygospores (Tucker 1981). The paraphyletic relationship between *Macrobiotophthora vermicola* and *Conidiobolus* s.l. was also revealed by Gryganskyi et al. (2012). In this paper, we treated it as a new combination and, therefore, proposed a monophyletic group of the new genus *Neoconidiobolus*.

In Clade I of the genus *Capillidium*, seven species grouped in a monophyletic clade with good support (100/1.00) and the synapomorph of producing capilliconidia: *Conidiobolus adiaeretus* (= *Capillidium adiaeretum*), *Co. bangalorensis* (= *Ca. bangalorensis*), *Co. denaeosporus* (= *Ca. denaeosporum*), *Co. heterosporus* (= *Ca. heterosprum*), *Co. lobatus* (= *Ca. lobatum*), *Co. pumilus* (= *Ca. pumilum*) and *Co. rhysosporus* (= *Ca. rhysosporum*). As a note, *Co. denaeosporus* was synonymised with *Co. pumilus* (King 1976b), but herein its taxonomic status of species level was accepted according to the phylogeny. *Co. adiaeretus* forms not only capilliconidia but also microspores (Callaghan et al. 2000).

In Clade II of the genus *Neoconidiobolus*, all 14 strains comprising 10 species produce neither microspores nor capilliconidia. Amongst these, *C. antarcticus* was identified as a synonym of *C. osmodes* (Chen and Huang 2018), which was confirmed here as they grouped into a robust clade.

Considering its long history and significant impact, we kept and emended the genus *Conidiobolus* and the original illustrations of the type species *C. utriculosus* (Brefeld 1884) were designated as its lectotype. Thus, we were able to recognise clade III under the genus name *Conidiobolus* on the basis of its synapomorph, namely microspores. In Clade III of the genus *Conidiobolus*, all species definitely produce microspores, except *Conidiobolus dabieshanensis*, *C. iuxtagenitus*, *C. khandalensis* and *C. lichenicolus*. Microspores have never been observed in *C. dabieshanensis* and *C. iuxtagenitus* (King 1977; Waters and Callaghan 1989; Nie et al. 2017), but cases for *C. khandalensis* and *C. lichenicolus* are somewhat different. For *C. khandalensis*, the protologue did not document any microspores (Srinivasan and Thirumalachar 1962b; King 1977), but they can be observed on 2% water-agar at 16 °C (Fig. 5h). Although the microspore of *C. lichenicolus* was not mentioned in the original description, the ability to produce microspores has been exhibited in accordance with original illustrations (Srinivasan and Thirumalachar 1968a). The phylogeny also resulted in the following taxonomic treatments. On the one hand, some previously synonymised taxa recover their specific status, for example, *C. gonimodes*, *C. megalotocus* and *C. mycophagus* should be separated from *C. incongruus*, *C. macrosporus* and *C. mycophilus*, respectively. On the other hand, *C. chlamydosporus* is synonymised with *C. firmipilleus*.

In Clade IV of the genus *Microconidiobolus*, *Conidiobolus undulatus* was identified as a synonym of *C. paulus* (= *M. paulus*) by King (1976b), which is supported by our molecular data. Otherwise, *C. nodosus* (= *M. nodosus*) and *C. terrestris* (= *M. terristris*) were classified as synonyms of *C. lachnodes* (= *Neoconidiobolus lachnodes*) in the study of King (1976b). Morphologically, *C. lachnodes* bears larger primary conidia (9–25 × 10–27 μm) than *C. nodosus* (13–16 × 17–22 μm) and *C. terrestris* (8–12 μm in width) (Drechsler 1955b; Srinivasan and Thirumalachar 1967, 1968a). Furthermore, *C. lachnodes* was located in Clade II and is distantly related to *C. nodosus* and *C. terrestris*. Therefore, *C. nodosus* and *C. terrestris* are accepted as two distinct species. This clade comprises four ex-type strains, all producing smaller primary conidia (mostly less than 20 μm) and can be morphologically easily distinguished from other *Conidiobolus* species.

Phylogenetically, *Conidiobolus lamprauges* does group with Clade III and received strong bootstrap support (100/1.00). Morphologically, this species produces small primary conidia (12.5–20 × 15–22 μm) without microconidia or capilliconidia and is similar to species within Clade IV. Its taxonomic status remains unclear in the present study.

Acknowledgements

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